can be time-ordered according to the DIMS trajectory and this ordering is essentially the same for forward and backward transitions. These results suggest that DIMS is capable of simulating realistic macromolecular transitions. From the simulated trajectories we can present a molecular detailed picture of a macromolecular transition. We discuss the conformational change of AdK with respect to the presence or absence of ligands, the relevance of salt bridges, and the motions of rigid domains.

[1] C. Vonrhein, G. J. Schlauderer, and G. E. Schulz. Movie of the structural changes during a catalytic cycle of nucleoside monophosphate kinases. Structure 3 (1995),483–490.

363-Pos Board B242

Conserved Protein Flexibility And Pathways Of Energy Flow In Enzyme Catalysis

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Conformational fluctuations in enzymes have significant affect on catalysis. Several enzymes show the presence of a network of coupled motions associated with the catalytic step. Here, we describe our recent studies to identify and characterize coupled motions in members of a diverse family of enzymes namely the dinucleotide binding Rossmann fold proteins (DBRP), sharing a common sub-step of hydride transfer from the dinucleotide cofactor to the substrate.

Results show that in spite of low sequence/structural homology, the overall intrinsic dynamical flexibility during the course of the enzyme reaction is conserved. These dynamical fluctuations span from the exterior surface regions to the active site of the protein and form pathways. These pathways are connected via hydrogen bonds/hydrophobic interactions, which are conserved across prokaryotes and eukaryotes alike.

In order to characterize the energy flow within these pathways, we use an integrated information theoretic and biophysical approach to study how energy may propagate within the DBRP super-family. The studies reveal for the first time how energy is propagated from the exterior flexible surface regions of the protein to the active site of the protein.

364-Pos Board B243

Accelerated Target Selection By Repair Enzymes Through Charge Transport

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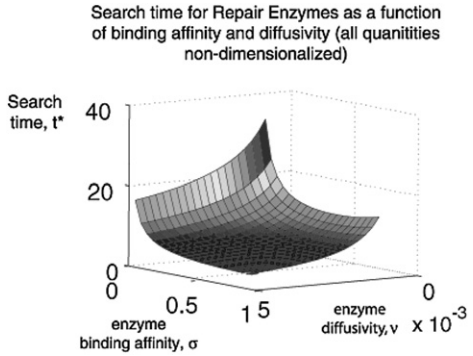
A Charge Transport (CT) mechanism has been proposed in several papers (for example see Yavin *et al.* PNAS **102**, 3546 (2005)) to explain the co-localization of Base Excision Repair (BER) enzymes to lesions (damaged bases) on DNA. The CT mechanism relies on the presence of iron-sulfur clusters on the enzymes; these clusters can undergo redox reactions to modify the enzymes' binding affinity. The redox reactions are mediated by the DNA strand and involve the exchange of electrons between individual BER enzymes. This process effectively increases the desorption rate of enzymes to promote their redistribution and co-localization to lesions.

We study the search times of BER enzymes to lesions by using a mass action model of enzyme dynamics and electron transport. We show that when the enzyme copy number is small, the CT mechanism reduces the search time of otherwise ''passive'' enzymes that simply attach to the DNA without desorbing. Other physical effects in our enzyme model include an explicit treatment of their dynamics in solution, diffusion along the DNA and facilitated adsorption by guanine radicals.



365-Pos Board B244

Reference-Free Identification of Dynamic Structural Domains in Proteins: Comparison of Numeric Predictions with NMR Measurements

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Understanding proteins' functionalities, which are intimately related with their structural conformations, require a robust characterization of conformational changes which occur in proteins in response to external impacts, as well as spontaneously. This presentation introduces a novel numeric methodology to identify dynamic structural domains in proteins, which is based on the recent theoretic invention [M. Stepanova, Phys. Rev. E76 (2007) 051918]. The methodology employs a fundamental, reference-free approach including identification of essential collective coordinates by the covariance analysis of molecular dynamics trajectories, construction of the Mori projection operator with these collective coordinates, and analysis of the corresponding generalized Langevin equations (GLE). The dynamic domains are identified as groups of atoms that show a dynamic coupling in the GLE. Since the methodology is based on a rigorous theory, the outcomes are physically transparent: the dynamic domains are associated with regions of relative rigidity, whereas off-domain regions are relatively soft. In the presentation, applications of the new structural analysis are demonstrated for the examples of protein G and prion proteins. Experimental NMR-based model-free S2 profiles, random coil indexes, and amplitude correlation data are compared with the numeric analysis, which includes (i) robust systems of dynamic structural domains and (ii) dynamically consistent local flexibility descriptors. It is shown that these numerical results agree well with the available NMR experiments. It is also demonstrated that the dynamic domains and the corresponding flexibility descriptors represent highly sensitive scores for characterization and comparison of proteins' conformations. Even very subtle changes in collective behaviors in macromolecules can be easily detected, visualized, and interpreted. The introduced methodology provides the community with a novel powerful tool for interpretation of NMR experiments, as well as for characterization, comparison, and dynamic analysis of proteins' conformational behaviors.

366-Pos Board B245

Spontaneous Substrate Binding and Formation of the Bound State in Glycerol-3-Phosphate Transporter (GlpT)

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GlpT is an antiporter mediating the uptake of glycerol-3-phosphate (G3P) across the membrane using preexisting gradient of inorganic phosphate (Pi). GlpT is believed to function through an alternating access mechanism, in which the two functional states inter-convert through rocker-switch type of conformational changes. However, the crystal structure of GlpT is only available in its cytosol-open state. Furthermore, the location of the binding site and residues involved in substrate binding are largely unknown. We have carried out an exhaustive set of long (50 ns or longer) molecular dynamics simulations of GlpT in the presence of all physiologically relevant substrates, i.e., monovalent and divalent Pi and G3P, as well as in the apo state as control. The substrate is placed at the opening of the lumen in the beginning of each simulation. In all of the simulations, we observe rapid, spontaneous binding of the substrate in less than 10 ns. All trajectories consistently yield a common binding pathway, composed of several conserved residues: K80 acting as a ''fishing hook'', one of the symmetrically positioned arginines (R45), and H165. The phosphate moiety of the substrate first binds to K80, which brings the substrate to a close contact with R45 and H165. Despite its symmetrical position to R45, no direct contact with conserved R269 is observed in any of the simulations. Neutralizing any one of the above residues impairs binding as revealed by additional simulations. Moreover, substrate binding results in appreciable closure of the helices in the cytoplasmic side illuminating initial steps of the rocker-switch mechanism. Our MD simulations reveal a common pathway involved in binding of the substrate, a detailed view of the binding site, and initial protein conformational changes induced by substrate binding.

367-Pos Board B246

Single Molecule Fret Reveals Novel Dynamic Structure And Stoichiometry Of L27 Domain-mediated Polarity Complexes Formed By Drosophila Sdt/DPatj/DLin-7

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Cellular differentiation is frequently regulated by multi-protein complexes where the spatial proximity of the components facilitates biological function. There is immense interest in isolating the individual components involved as

well as determining how their interaction regulates differentiation. Multiparameter fluorescence detection nowadays allows direct observation of molecular processes at the single molecule level.
The obtained evolutionary conserved multi-protein complex is located in the subapical region (SAR) of embryonic epithelia and plays a central role in the maintenance of epithelial cell polarity, morphogenesis and survival of photoreceptor cells in Drosophila melanogaster. The complex is composed of the four proteins DPATJ and DLin-7, Stardust (Sdt), and Crumbs (Crb). The scaffold protein Sdt contains two subsequent L27 modules, which mediate the interaction with DPATJ and DLin-7 through their L27 domains. The trans-membrane protein Crb binds to the PDZ domain of Sdt with its cytoplasmic tail.
Here we use single-molecule fluorescence resonance energy transfer (FRET) in order to understand better quantitative parameters and spatial dynamics of the complex. We show that free DLin-7 exists in two major conformations, a high-FRET, folded state, and a second low FRET, unstructured state, which allows us to assign the L27 domain in DLin-7 as an "extended" disordered region.
Upon formation of a complex with Stardust, the proportion of folded DLin-7 molecules increases. Depending on the Stardust/DLin-7 ratio different heteromers are formed. The presence of DPATJ further increases the fraction of DLin-7 bound to Stardust, indicating that the L27-domains of all three proteins contribute to a positive cooperativity. Thus, L27 domains are versatile modules ideally suited to provide flexibility of protein complexes.

368-Pos Board B247
Conformational Flexibility of the GM2 Activator Protein Loop Regions Investigated By Site Directed Spin Labeling EPR Spectroscopy
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The GM2 activator protein (GM2AP) is an essential component in the degradation pathway of neuronal gangliosides. GM2AP is a required accessory protein for the hydrolytic conversion of GM2 to GM3 by a water soluble hydrolase. The X-ray structure of GM2AP reveals a β-cup topology with multiple conformations of the protein within the unit cell. Because the crystal structures show different conformations of the putative membrane binding loops, we have utilized site-directed spin labeling to investigate conformational flexibility of these loops for protein in solution and bound with GM2 ligand. As such, a series of single and double CYS mutants (still with original 8 CYS in 4 disulfide bridges) have been generated and spin labeled with MTSL. EPR spectra of spin labeled GM2AP were collected with and without GM2 ligand, and no significant changes in the EPR lineshape were seen. EPR spectra were simulated for spin labels located in the loop regions and reveal multiple component fits, while those in the backside of the β-cup beta strands have single component fits. For certain sites in the mobile loops, spectra were acquired as a function of temperature. From these lineshape simulations, the activation energy for the conformational change has been determined. The SDSL EPR results indicate that the multiple conformations observed in the crystallographic unit cell are populated in solution and represent conformational flexibility of the protein; which is not necessarily altered by binding to lipid ligands. Additionally, spin labeled protein was analyzed by mass spectrometry to confirm proper formation of the four disulfide linkages and addition of only one spin label at the mutant cysteine.

369-Pos Board B248
Single-Molecule Protein Conformational Dynamics and Molecular Interaction Dynamics under Enzymatic Reactions
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Enzymatic reactions are traditionally studied at the ensemble level, despite significant static and dynamic inhomogeneities. Subtle conformational changes play a crucial role in protein functions, and these protein conformations are highly dynamic rather than being static. Protein-molecular interactions define the enzymatic reaction potential surface, pathway, and dynamics. The single-molecule protein-protein interaction dynamics reveals the nature of the molecular complex formation and recognition that are critical for an enzymatic reaction to occur. We applied AFM-enhanced single-molecule spectroscopy to study the mechanisms and dynamics of enzymatic reactions involved with kinase and lysozyme proteins. Enzymatic reaction turnovers and the associated structure changes of individual protein molecules were observed simultaneously in real-time by single-molecule FRET detections. We obtained the rates for single-molecule conformational active-site open-close fluctuation and correlated enzymatic reactions. We have demonstrated a specific statistical analysis to reveal single-molecule FRET anti-correlated fluctuations from a high background of fluorescence correlated thermal fluctuations. Our new approach is applicable to a wide range of single-molecule FRET measurements for protein conformational changes under enzymatic reactions.

370-Pos Board B249
Slippage Between Noncovalently Bound Filaments Of Self-assembling Peptide
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Much attention has been given to the rupture of noncovalent chemical bonds in protein dynamics, and Bell's model has become widely used. In many cases, however, unbinding or unfolding requires that multiple energy barriers be overcome in parallel, in coordinated failure events. We examine one such system, slippage between ß-sheet filaments of the self-assembling peptide RAD16-II ([RARADADA]2). RAD16-II forms amphiphilic ß-sheet filaments, with alanine side chains forming the hydrophobic surface. In an aqueous environment, filaments are found in pairs, with their hydrophobic faces placed together. We examine slippage between two filaments using steered molecular dynamics simulations. We observe that alanine side chains on one ß-sheet filament form a rectangular array, and the alanine side chains of the opposing sheet occupy the interstices. For slippage to occur, these methyl groups must jump from one interstice to the next. Since the alanines in one ß-sheet are elastically linked, this failure occurs in a cooperative manner. Slippage of a single alanine side chain correlates with slippage of its immediate neighbors, and a dislocation propagates across the bound surface within a few picoseconds. We present a one-dimensional, coarse-grained model based on Langevin dynamics, that incorporates the basic elements of this system: multiple elastically linked particles each residing in an energy well and overcoming an energy barrier under applied force. The coarse-grained model shows good agreement with molecular dynamics results and provides a useful platform for studying coordinated failure events. [Supported by the NHLBI, EB003805.]

371-Pos Board B250
Multiple Channels of Structural Relaxations in Functional Proteins
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We elucidate the physics of the dynamical transition via 15 ns long molecular dynamics simulations at a series of temperatures (spanning 160 - 280 K) where the protein retains its native structure. By tracking the energy fluctuations, we show that the protein dynamical transition is marked by a cross-over from a piecewise stationary to stationary set of processes that underly the dynamics of protein motions in the water environment.
We find that a two-time-scale function captures the non-exponential character of backbone structural relaxations. One is attributed to the collective protein motions and the other to local relaxations. The former is well-defined by a single-exponential, nanosecond decay that is operative at all temperatures. The latter, on the other hand, is described by a large number of single-exponential motions that display a distribution of time-scales. Though their average remains on the order of 10 ps at all temperatures, the distribution markedly contracts with the onset of the dynamical transition. Interestingly, the collective motions are shown to impose bounds on the time-scales spanned by the local dynamical processes, although they are not directly involved in the transition.
The piecewise stationary character below the transition implicates the presence of a collection of sub-states whose inter-communication is restricted. The ineffectiveness of these sub-states to influence the overall relaxation time is shown to require a wide distribution of local motion time-scales, extending well beyond that of nanoseconds. At physiological temperatures, on the other hand, local motions are confined to time-scales faster than nanoseconds. This relatively narrow window makes possible the appearance of multiple channels for the backbone dynamics to operate, providing alternative routes for protein functionality.

372-Pos Board B251
Solvent Bridging Determines The Molecular Architecture Of The Unfolding Transition State Of A Protein
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Protecting osmolytes are ubiquitous in nature, where they play a vital role stabilizing intracellular proteins against adverse environmental conditions. While the solution thermodynamics of protein/osmolyte mixtures has been well characterized, information is lacking on how osmolytes influence the transition state structure and dynamics of proteins. Here we demonstrate a combination of single molecule force-clamp spectroscopy and solvent substitution that directly identifies the role of protecting osmolytes in the unfolding transition state structure of a protein. We measure the effect of osmolyte substitution on the rate of forced unfolding the I27 titin module. From the force dependency of the unfolding rate for each osmolyte we determine ΔG_U, the height